

Please replace the paragraph beginning at page 30, line 2 with the following:

310
--A fused gene was prepared comprising the sequence encoding Synthetic "Core" Streptavidin (Thompson and Weber 1993) fused to a PPI-II signal sequence. The Streptavidin cDNA, carried on the plasmid pET3a was cloned into the EcoR I/Xba I sites of pUC 19 (Fig. 3). The PPI-II signal sequence (Fig. 4) which contains an intron was isolated from recombinant plasmid using PCR with a sense primer binding to pUC19 and an antisense primer incorporating an EcoR I site into a 5' overhang. The primers were as follows.

sense primer (SEQ ID NO:17):

5' - CTG CAG GTC GAC TCT AGA GGA - 3'

antisense primer (SEQ ID NO:18):

5' - GGT GAA TTC TTA GTA CAG ATC TTC GCA - 3'--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 8, at the end of the application.

REMARKS

In accordance with 37 C.F.R. §§1.821 to 1.825, Applicants request entry of this amendment. This amendment is accompanied by a floppy disk containing SEQ ID NOS:1-34, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.



CHRISTELLER et al.
Application No.: 09/743,690
Page 6

PATENT

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Kenneth A. Weber
Reg. No. 31,677

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
KAW:dmw

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 9 of page 6 has been amended as follows:

Figure 1 shows the nucleic acid sequence of Potato Proteinase Inhibitor I (PPI-I/pUC19) (SEQ ID NO:1). The signal sequence is in bold type and the start and stop codons are in italic. The mutagenic primer is denoted by underlined in lower case with the Bgl II site created by mutagenesis in bold italic. The upstream and downstream primers used were the Forward and Reverse M13(lacZ) Primers [Perkin Elmer].

Paragraph beginning at line 15 of page 6 has been amended as follows:

Figure 2 shows Avidin cDNA (pGEMav) (SEQ ID NO:2). The signal sequence represented in bold type, start and stop codons are in italic, primers are underlined lower case with the BamH I site created by mutagenesis in italic. The downstream primer used was the Reverse M13(lacZ) Primer [Perkin Elmer].

Paragraph beginning at line 20 of page 6 has been amended as follows:

Figure 3 shows streptavidin cDNA (Streptavidin/pUC19) (SEQ ID NO:3). Start and stop codons are in bold type. EcoR I and Xba I sites are in italic.

Paragraph beginning at line 23 of page 6 has been amended as follows:

Figure 4 shows potato proteinase inhibitor II (PPI-II/pUC19) (SEQ ID NO:4). The signal sequence is represented in bold type and start and stop codons are in bold italic.

Underlined type denotes the intron within the signal sequence. The asterisk denotes the result of PCR error during isolation of the PPI-II sequence.

Paragraph beginning at line 5 of page 7 has been amended as follows:

Figure 7 shows a schematic representation of the pART7 expression cassette as it was cloned into the pART27 binary vector; A) containing the PPI-I-Avidin gene fusion and B) containing the PPI-II/Streptavidin gene fusion (altered BamH I site = SEQ ID NO:5).

Paragraph beginning at line 9 of page 7 has been amended as follows:

Figure 8 shows PPI-I/Avidin gene fusion sequence (SEQ ID NO:6) (A) and fusion protein sequence (SEQ ID NO:7) (B): The fusion protein has a total of 161 amino acids; the PPI-I sequence is represented by italic type with bold type denoting the PPI-I signal peptide. Two amino acids, novel to both the PPI-I and the Avidin peptide sequences and represented in lower case, were introduced with the ligation of the Bgl II and BamH I compatible cohesive ends.

Paragraph beginning at line 15 of page 7 has been amended as follows:

Figure 9 shows PPI-II/Streptavidin gene fusion sequence (SEQ ID NO:8) (A) and fusion protein sequence (SEQ ID NO:9) (B): The fusion protein has a total of 168 amino acids; the PPI-II sequence is represented by italic type with bold type denoting the PPI-II signal peptide. Three amino acids, novel to both PPI-II and the Streptavidin peptide sequences and represented in lower case, were introduced at the point of fusion.

Paragraph beginning at line 24 of page 7 has been amended as follows:

Figure 12 (A) shows the nucleotide sequence for the gene for streptavidin (SEQ ID NO:10) (Argarana *et al.* 1986). The signal sequence is represented in bold type, start and stop codons in bold italic. (B) shows the protein sequence for streptavidin (SEQ ID NO:11). The signal sequence is represented in bold type.

Paragraph beginning at line 7 of page 28 has been amended as follows:

Primers:

Forward M13 (lacZ) Primer [Perkin Elmer] (SEQ ID NO:12):

5'-GCCAGGGTTTTCCCAGTCACGA-3'

Reverse M13 (lacZ) Primer [Perkin Elmer] (SEQ ID NO:13):

5'-GAGCGGATAACAATTTACACAGG-3'

Avidin Upstream Primer (SEQ ID NO:14):

5'-GCACACCCGGCTGTCCACCTG-3'

Phosphorylated Mutagenic Primers

PPI-I mutagenic primer (SEQ ID NO:15):

5'-PGATGGACCAGAGATCTTAGAAC-3'

Avidin mutagenic primer (SEQ ID NO:16):

-
5'-PGGCTCCCGGGATCCCTGCCAG-3'

Paragraph beginning at line 2 of page 30 has been amended as follows:

A fused gene was prepared comprising the sequence encoding Synthetic "Core" Streptavidin (Thompson and Weber 1993) fused to a PPI-II signal sequence. The Streptavidin cDNA, carried on the plasmid pET3a was cloned into the EcoR I/Xba I sites of pUC 19 (Fig. 3). The PPI-II signal sequence (Fig. 4) which contains an intron was isolated from recombinant plasmid using PCR with a sense primer binding to pUC19 and an antisense primer incorporating an EcoR I site into a 5' overhang. The primers were as follows.

sense primer (SEQ ID NO:17):

5' - CTG CAG GTC GAC TCT AGA GGA - 3'

antisense primer (SEQ ID NO:18):

5' - GGT GAA TTC TTA GTA CAG ATC TTC GCA - 3'